REMARKS

Applicants' representative wishes to thank the Examiner for his comments made prior to submitting this amendment.

Claims 3, 4, 6-15, and 21-24 remain in the application. Claims 1, 2, 5, and 16-20 are cancelled by this amendment without prejudice. Claims 22-24 are added by this amendment.

The paragraph beginning at page 5, line 19 is amended to remove the definition of "amino acid substitution," which was objected to. The paragraph is also amended to remove the definition of "stabilizing amino acid substitution," which was objected to.

Claims 3 and 9 are amended to change the term "stabilizing amino acid substitution" to "one or more terminal histidine residues." This incorporates limitations from cancelled claim 5 and claim 21 into both claims. Support is also found at page 7, lines 6-10.

Claims 3 and 9 are amended to add the limitation that the bound enzyme is catalytically active. Support for this amendment is found in the now cancelled definition of "stabilizing amino acid substitution" and at page 4, lines 16-19, and page 12, lines 7-11. Applicants contend that this amendment should be entered because the matter was addressed in Applicants' prior response to the first rejection and in the Examiner's final rejection.

The Examiner discussed the definition of a "stabilizing amino acid substitution" as "a substitution that non-covalently bonds to IDA salts or NTA salts without substantially effecting the catalytic function of the enzyme," and stated that the "definition makes no reference that the 'bound' enzyme must be catalytically active." Page 11, lines 4-10. (As amended in this response, the "substitution" is one or more terminal histidine residues.) The Examiner's interpretation of the definition appears to apply two different meanings to the word "substitution." In reference to binding to salts, the Examiner's interpretation of "substitution" seems to be a portion of the enzyme. This portion or substitution is capable of binding to the salt. In reference to catalytic function, the Examiner's interpretation of "substitution" seems to be the act of changing residues and the result thereof. The act of substitution alone does not affect function. Under this interpretation, the definition does not state that the bound enzyme is catalytically active. However, a more reasonable interpretation uses a consistent meaning of "substitution."

The word "substitution" in the definition refers to the altered portion of the enzyme, not to the act of making a substitution. The definition states that this portion of the enzyme binds to

salts without affecting catalytic function. Thus, the binding, as opposed to the act of substitution, does not affect the catalytic function. In other words, the bound enzyme is catalytically active.

For the sake of clarity and to comply with objections, this amendment cancels the term "stabilizing amino acid substitution" and its definition, and adds the equivalent language of "the bound enzyme is catalytically active." As the meaning is the same, this amendment does not raise a new issue. Further, the Examiner stated his likely reaction to the inclusion of a limitation that bound enzyme is catalytically active. It appears that the issue was considered by the Examiner in the final rejection.

Claim 21 is amended to remove the term "stabilizing amino acid substitution."

New claims 22 and 23 depend from claim 9 and correspond to claims 6 and 15, which depend from claim 3.

New claim 24 depends from claim 3 and corresponds to claim 21, which depends from claim 9.

Reconsideration of the application in view of the above amendment and the following arguments is requested.

The Examiner objected to the amendment of 09/18/2002 for incorporation of new matter by reciting, "A 'stabilizing amino acid substitution' is a substitution that non-covalently bonds to IDA salts or NTA salts without substantially effecting the catalytic function of the enzyme." This amendment cancels the previous amendment without prejudice.

The Examiner objected to the amendment of 09/18/2002 for the definition of the term "amino acid substitution" as including both the addition of one or more amino acid residues to a protein without removing any residues as well as changing one or more residues in a protein to other residues. The Examiner stated that this definition was repugnant to the usual meaning of the term substitution. This amendment cancels this definition without prejudice.

Claim rejections – 35 U.S.C. § 112

The Examiner rejected claims 3-21 under 35 U.S.C. 112, second paragraph as being indefinite for use of the term "amino acid substitution" as defined in the amendment of 09/18/2002. This amendment cancels this term from claims 3 (4, 6-8, 15, and 24 dependant

thereon) and 9 (10-14 and 21-23 dependent thereon). The basis for the rejection has been removed.

The Examiner rejected claims 17 and 20 under 35 U.S.C. 112, second paragraph as being indefinite for the recitation "known to be innocuous to the function of the enzyme." This amendment cancels these claims. The basis for the rejection has been removed.

The Examiner rejected claims 16, 17, 19, and 20 under 35 U.S.C. 112, second paragraph as being indefinite for the recitation "at a binding site on the enzyme." This amendment cancels these claims. The basis for the rejection has been removed.

The Examiner rejected claims 3-21 under 35 U.S.C. 112, first paragraph for lack of written description. The limitation that the enzyme is genetically engineered to include one or more terminal histidine residues has been incorporated into claims 3 (4, 6-8, 15, and 24 dependant thereon) and 9 (10-14 and 21-23 dependent thereon).

The Examiner stated that the only example of genetically engineering an enzyme is to add histidine residues to the amino terminus of *E. coli* thioesterase 1 and that this example does not convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. By this amendment, the claims have been limited to genetically engineering an enzyme to include one or more terminal histidine residues. Although only one working example is disclosed, the application discloses that the invention may be practiced with other enzymes including thioesterases as a class, lipase, and enzymes useful for destroying waste materials (p. 5, lines 19-22). Methods for genetically engineering terminal histidine into any desired enzyme are known in the art, including those methods disclosed at p. 6, line 20-p. 7, line 7. Further, it is disclosed that the main criterion for the process to be effective is that the binding site on the enzyme (the location of the terminal histidine) be far away from or innocuous to the function of the enzyme's catalytic site (p. 6, lines 12-14). The structure of an enzyme can be determined to find out whether the termini are far away from or innocuous to the function of the enzyme's catalytic site. It is not necessary to disclose additional enzymes with terminal histidine in order to show possession of the invention.

The Examiner rejected claims 3-20 under 35 U.S.C. 112, first paragraph for lack of enablement. There was no enablement rejection of claim 21. This amendment adds the limitation of one or more terminal histidine residues into claims 3 (4, 6-8, 15, and 24 dependant

thereon) and 9 (10-14 and 21-23 dependent thereon). Addition of one or more terminal histidines is enabled at page 7, lines 6-10. All pending claims are enabled by the disclosure.

Claim rejections – 35 U.S.C. § 102

The Examiner rejected claims 9-12, 14, and 19-21 under 35 U.S.C. § 102(b) as being anticipated by Qiagen Product Guide. In response to Applicants' arguments of 09/18/2002, the Examiner stated that the definition of "stabilizing amino acid substitution" does not state that the enzyme is catalytically active while bound to the salt. This definition has been canceled and a limitation that "the bound enzyme is catalytically active" has been added to claims 3 and 9.

The Examiner also noted that the inclusion of a limitation that the bound enzyme must retain catalytic activity while bound would likely result in an obviousness rejection, because Qiagen teaches that a 6xhis tag rarely affects protein structure or function and need not be removed from the purified protein. However, Qiagen is referring to activity of the purified protein that has been eluted from the column. Qiagen does not disclose whether the enzyme has any catalytic activity while it is bound to the column. As this limitation is not found in the prior art, an obviousness rejection would not be warranted.

Claim rejections – 35 U.S.C. § 103

The Examiner rejected claim 13 under 35 U.S.C. § 103 as being obvious over Qiagen in view of Lu. Claim 9, upon which claim 13 depends, has been amended to include the limitation that "the bound enzyme is catalytically active." As explained above, this limitation is not disclosed in Qiagen. Neither is it disclosed in Lu. Qiagen and Lu cannot be combined to produce the invention of claim 13.

The Examiner rejected claims 3-5, 7, 8, and 15-18 under 35 U.S.C. § 103 as being obvious over Singh, LeJeune, and Polayes. The Examiner has stated a motivation for combining these references, however, this motivation is not found in the references.

The recent case *In re Lee*, 61 U.S.P.Q.2d 1430, 277 F.3d 1338 (Fed. Cir. 2002) reviewed the requirements for finding a motivation to combine references. In that case, the Board of Patent Appeals and Interferences had held that the "conclusion of obviousness may be made from common knowledge and common sense of a person of ordinary skill in the art without any

specific hint or suggestion in a particular reference." *Id.* at 1432. The Federal Circuit reversed, citing a string of precedent.

> When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness. ... [T]here must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant. ... "[P]articular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed." ... [T]he examiner can satisfy the burden of showing obviousness of the combination "only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references." (*Id.* at 1433-1434, citations omitted.)

The court held that the Board may not reject the need for "any specific hint or suggestion in a particular reference." *Id.* at 1434. In the present application, the Examiner has stated motivations to combine the references, but has not cited to a "specific hint or suggestion" found in one reference that would lead one of ordinary skill in the art to select another reference and combine them. Rather, the motivation is found in the present application.

In the office action of 06/18/2002, the Examiner stated that one of ordinary skill in the art at the time of filing would have been motivated to immobilize a nerve agent hydrolyzing enzyme, such as phosphotriesterase, as a method of enhancing the stability of the enzyme as taught by LeJeune (p. 12, lines 16-18). There is not a citation to the prior art as the source of this motivation, other than to LeJeune. However, LeJeune is also the source of the same teaching. The only motivation found in LeJeune is to do what LeJeune teaches. Nothing is cited from LeJeune (or any other reference) that would motivate one of ordinary skill to select Singh or Polayes and combine it with LeJeune. The same reasoning applies to the other motivations stated by the Examiner.

The only motivation cited that may lead to another reference is that "Singh teach the use of an enzyme which contains several exposed histidine residues. ... However, not all enzymes have the necessary number of exposed histidine residues, hence the motivation to add a polyhistidine tail as taught by Polayes et al." (Office action of 12/08/2002, p. 15, lines 13-19.)

However, Singh only refers to "selection of an enzyme" (col. 23, line 39) and not engineering of an enzyme. The desirability of a polyhis tail in particular is not suggested. Further, Polayes is directed to purification of a protein and not immobilization; and Polayes cleaves the polyhis from the protein, which would be detrimental to Singh. One of ordinary skill in the art would not have selected Polayes for combining with Singh. Finally, no motivation in the prior art is cited to also combine with LcJeune.

Although the Examiner stated a basis for a reasonable expectation of success (Office action of 06/18/2002, p. 13, lines 6-12), this does not substitute for the motivation. They are two separate requirements. "The first requirement is that a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential evidentiary component of an obviousness holding.' ... The second requirement is that the ultimate determination of obviousness 'does not require absolute predictability of success. ... All that is required is a reasonable expectation of success.'" *Brown & Williamson Tobacco Corp. v. Phillip Morris, Inc.*, 56 U.S.P.Q.2d 1456, 1459, 229 F.3d 1120 (Fed. Cir. 2000) (citations omitted). Further, the Examiner has not made a finding as to the level of skill in the art.

In view of the foregoing, it is submitted that the application is now in condition for allowance.

In the event that a fee is required, please charge the fee to Deposit Account No. 50-0281,

and in the event that there is a credit due, please credit Deposit Account No. 50-0281.

Respectfully submitted,

John J. Karasek
Reg. No. 36,182
Phone No. 202-404-1552
Associate Counsel (Patents)
Naval Research Laboratory
4555 Overlook Ave, SW
Washington, DC 20375-5325

Prepared by:
Joseph T. Grunkemeyer
Reg. No. 46,746
Phone No. 202-404-1556

CERTIFICATION OF FACSIMILE TRANSMISSION

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3/12/03
Date

Joseph T. Grunkemeyer

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The specification has been amended as follows:

Paragraph beginning at page 5, line 19

Examples of enzymes which are useful in detoxifying nerve agents are thioesterases, although the process of the present invention can be used with any type of enzyme useful for destroying waste materials. One example of this is lipase, which is used for digesting waste onboard ships. The enzymes are genetically engineered to include a poly-His tail as well as other stabilizing amino acid substitutions. [As used herein, the term "amino acid substitution" includes both the addition of one or more amino acid residues to a protein without removing any residues as well as changing one or more residues in a protein to other residues. A "stabilizing amino acid substitution" is a substitution that non-covalently bonds to IDA salts or NTA salts without substantially effecting the catalytic function of the enzyme.] Non-covalent enzyme immobilization on polymerized liposomes was effected by co-polymerizing amphiphiles containing metal salts of iminodiacetic acid or nitrilotriacetic acid with other polymerizable amphiphiles and then binding the enzyme to the iminodiacetic acid-metals or NTA-metal salts on the outer surfaces of the vesicles. This technique relies on the strong binding affinity between iminodiacetate salts or NTA salts and polyhistidine, which has been made available on the surface of the enzyme selected for immobilization through genetic engineering. The enzymes that can be used for this technique are those enzymes that have appropriately reactive surface available histidines or which have a histidine tag that can be added through site specific mutagenesis. This includes, or course, polyhistidine. Histidine forms a strong bond with iminodiacetic acid salts, such as copper, zinc, cobalt, and nickel iminodiacetate salts, and nitrilotriacetic acid salts, such as copper, zinc, cobalt, and nickel salts. The main criterion for this process to be effective is that the binding site on the enzyme be far away from or innocuous to the function of the enzyme's catalytic site. While silica is the preferred inorganic surface because it is relatively inexpensive and its properties are well understood, any type of metal oxide ceramic particles that can be formed similar to the Stober process starting with a metal alkoxide precursor can be used. Other types of inorganic surfaces that can be used in the process of the present invention include alumina, baria, titania, and zirconia.

The claims have been amended as follows:

Claims 1 and 2 have been cancelled.

3. (Twice amended) A method for stabilizing enzymes comprising:

genetically engineering an enzyme to include one or more terminal histidine residues [a stabilizing amino acid substitution];

copolymerizing an amphiphile containing a salt selected from the group consisting of metal salts of iminodiacetic acid, nitrilotriacetic acid, and mixtures thereof with other polymerizable amphiphiles to form vesicles; and

binding the genetically engineered enzyme to the salts on the outer surface of the vesicles;

wherein the bound enzyme is catalytically active.

Claim 5 has been cancelled.

9. (Twice amended) A method for stabilizing enzymes comprising:

genetically engineering an enzyme to include one or more terminal histidine residues [a stabilizing amino acid substitution]; and

attaching the [stabilized] enzyme to salt groups selected from the group consisting of metal salts of iminodiacetic acid, metal salts of nitrilotriacetic acid, and mixtures thereof on the surface of a particulate inorganic carrier;

wherein the bound enzyme is catalytically active.

Claims 16-20 have been cancelled.

21. (Amended) The method of claim 9, wherein the enzyme includes [stabilizing amino acid substitution is] a terminal polyhistidine chain.

Claims 22-26 are new.